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Developmental Pathoconnectomics and Advanced Fetal MRI

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Abstract: Developmental pathoconnectomics is an emerging field that aims to unravel the events leading to and outcome from disrupted brain connectivity development. Advanced magnetic resonance imaging (MRI) technology enables the portrayal of human brain connectivity before birth and has the potential to offer novel insights into normal and pathological human brain development. This review gives an overview of the currently used MRI techniques for connectomic imaging, with a particular focus on recent studies that have successfully translated these to the in utero or postmortem fetal setting. Possible mechanisms of how pathologies, maternal, or environmental factors may interfere with the emergence of the connectome are considered. The review highlights the importance of advanced image post processing and the need for reproducibility studies for connectomic imaging. Further work and novel data-sharing efforts would be required to validate or disprove recent observations from in utero connectomic studies, which are typically limited by low case numbers and high data drop out. Novel knowledge with regard to the ontogenesis, architecture, and temporal dynamics of the human brain connectome would lead to the more precise understanding of the etiological background of neurodevelopmental and mental disorders. To achieve this goal, this review considers the growing evidence from advanced fetal connectomic imaging for the increased vulnerability of the human brain during late gestation for pathologies that might lead to impaired connectome development and subsequently interfere with the development of neural substrates serving higher cognition.

Key Words: brain development, connectomics, diffusion tensor MRI, fetal MRI, functional MRI

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A central endeavor in neuroscience is to disentangle the complexity of the human brain and understand how groups of neurons interact with each other and integrate information under normal or pathological conditions. One way to study brain complexity is by characterizing the connectome, which is a comprehensive anatomical map of systems-level neuronal wiring. As the in- and outgoing connections determine the regions between which information can be exchanged,[1] long-range connectivity has paramount importance in governing brain functions. The (macro-)connectome reflects long-range connectivity, providing information about how the brain integrates information across spatially and functionally segregated cortical areas.[2,3]

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To date, the comprehensive, whole brain level mapping of connectivity remains an elusive and technically challenging task in humans as well as in larger primates, mainly due to brain size and wiring complexity. Despite this, the field of connectomic research has progressed greatly since the invention of in vivo neuroimaging techniques such as diffusion magnetic resonance imaging (MRI) for mapping structural connections,[4–6] or functional MRI (fMRI) for functional connectome mapping.[7] Although MRI has been mostly recognized as a clinical diagnostic tool, it has attracted increasing attention from developmental neuroscientists over the last 2 decades, mainly due to certain beneficial properties such as its noninvasive nature and applicability to sensitive human populations, such as infants or children. Indeed, connectomic research has been given fresh impetus by recent, large-scale neuroimaging initiatives, such as the Human Connectome Project,[8] Developing Human Connectome Project, or the UNC/UMN Baby Connectome Project.[9,10]

This issue of Topics in Magnetic Resonance Imaging is dedicated to diverse, exciting subjects in advanced fetal MRI, including connectome imaging. Since the 2000s, the vast majority of the scientific literature on human connectome imaging focused on the postnatal period or imaging prematurely born infants, and to date, the prenatal application of this method had marginal importance in clinical research with only a few specialized imaging centers performing advanced fetal diffusion MRI (dMRI) or fMRI. This review focuses on *developmental pathoconnectomics* with advanced fetal MRI, an emerging field that aims to unravel the events leading to (and outcome from) disrupted brain connectivity development. In the broader sense, the field covers works looking at the links between fetal and maternal distress and impaired connectivity development as well as disorders where malformation or maladaptive development of cerebral connectivity might play a central role in pathogenesis. Importantly, prenatal imaging may provide further evidence for the ''multiple-hit theory'' of pathogenesis, such as in congenital heart defects, where cerebral injury most likely results from a mixture of prenatal, perinatal, and postnatal (and hospitalization-related) events.

This review will start with an overview of the currently used acquisition techniques for connectomic imaging, with a particular focus on studies that have successfully translated these to the in utero or postmortem fetal setting. In order to provide a basic introduction to brain development for scientists with diverse medical backgrounds, a separate chapter will elucidate the biological mechanisms of normal and impaired connectome development. While the term *pathoconnectomics* suggests a focus on diseases, a short overview of recent articles employing advanced fetal MRI in normal (unaffected) neurodevelopment is provided. The latter aspect is crucial for the identification of prenatal periods where the central nervous system has increased vulnerability for pathologies. As the majority of the literature on whole-brain structural and functional connectomics relies on 2 main types of MRI techniques, namely dMRI and blood oxygen level dependent (BOLD) contrast fMRI, structural and functional connectivity will be discussed separately. A separate chapter will summarize the most pressing technical challenges and limitations confounding the interpretation of connectivity imaging data during development. An important goal of this article is to

give an updated overview of the works from the last decade that utilized advanced fetal MRI for structural and functional connectome mapping. On the basis of these works, the current evidence for the value of fetal MRI in detecting impaired connectivity development will be collected and presented.

ADVANCED FETAL MRI METHODS FOR MAPPING THE BRAIN CONNECTOME

Diffusion-weighted imaging (DWI) is a noninvasive MRI technique that probes the microscale displacement of water molecules, offering a unique insight into the organization of tissues.[11] Diffusion tensor MRI (DTI) provides increased sophistication compared with DWI, as it resolves the directional dependence of the water diffusion, and hence provides a depiction of the white matter morphology through sampling the magnitude and orientation of tissue diffusion anisotropy.[12] DWI and DTI belong to the larger family of MR sequences relying on diffusion sensitization (referred to as dMRI), which gained popularity for connectomic imaging in the last decade, as the only in vivo MRI method for mapping the structural brain connectome. Initial studies confirmed that dMRI is possible in vivo in utero,[13–17] even in the absence of myelinated fiber tracts, as a central assumption is that the spatially anisotropic restriction of diffusion is predominantly caused by the axonal membranes. In utero DTI studies utilized at least 12 to 15 diffusion weighting directions, whereas b-values were lower than in adult studies, and typically ranged from 500 to 1000 s/mm^2, except for the postmortem imaging of fixed brain specimen. Voxel size and slice thickness depend greatly on the scanner specifications, typical dimensions were 1 to 2 mm, and slice thickness is 2 to 5 mm. Postmortem DTI studies utilized high field strengths (3.0 to 4.7T) and the images had better spatial and angular resolution.[18–22] Table 1 gives an overview of the DTI protocols used by prenatal in utero and postmortem studies, focusing on works that carried out tractography or whole-brain connectivity mapping. Works that reported the microstructural (DTI-based) properties of the developing fetal brain without tractography or connectomic analysis and works that focused on the reproducibility of DTI were not listed. The first step during the estimation of the structural connectome from dMRI data is to trace fiber trajectories and quantify the probability of connections between brain regions via these routes. The latter is most simply done by counting the number of virtual ''streamlines'' connecting 2 regions.[23] A substantial departure from this method was the development of a diverse family of mathematical models that describe the relationship between the dMRI signal and the underlying distribution of fibers, resulting in improved outlines of anatomically reliable trajectories based on this data (for a review on tractography techniques and their inherent challenges in reconstructing the connectome, see Jelescu and Budde, 2017[24] and Maier-Hein et al[25]). In the majority of works, this analysis is performed after whole-brain or whole-cortex fiber tracing. Although diffusion tensor MRI and tractography is possible in utero,[13] the literature has so far been dominated by works describing the structural connectome of infants born prematurely, and only a few studies utilized whole-brain tractography for analyzing the developing fetal brain.[16,21]

Functional brain connectomes are constructed by calculating the functional connectivity strength between brain regions, which is typically measured as the statistical correlation or covariance of low-frequency signal fluctuations during resting-state BOLD fMRI.[7,26] The first fetal BOLD-fMRI experiment was conducted in 1999 by Hykin et al.[27] In utero conditions do not allow for cooperation of the subject, which limits stimulus modes to a minimum, for example, very basic visual,[28] auditory,[29] or vibroacoustic tasks.[30] Recent experiments using fMRI revealed functional activity patterns in the fetal brain without external stimuli with imaging times ranging from 1 minute to 10 minutes.[31–35] It is common practice in research groups utilizing fetal fMRI to have standard BOLD sequences; however, it is advisable to adjust the echo time to the changing relaxation time of the developing fetal brain (eg, typical scan: 50 ms, fetal brain: 100 to 120 ms advised[36]). This review focuses on works that utilized stimulus-free fMRI and carried out functional connectomic analysis. The commonly used imaging protocols are detailed in Fig. 1.

An important step in the analysis of fMRI data is the identification of the non-neural contribution of the MRI signal, such as motion effects, breathing and circulation-related low-frequency oscillations, and hardware noise. During fetal MRI, there is also a possible confounding effect from the surrounding maternal organs and amniotic fluid.

Although the term connectome comprises very broad spatial and temporal scales of neural connections (see review by Budd

TABLE 1. Summary of the Commonly Used DTI Acquisition Protocols for Fetal Tractography and Connectomic Analysis

Study	Aim	Field Strength	Sequence, TE/TR	Diffusion-weighting Directions	b-value	Voxel Dimension and Slice Thickness	Acquisition Time
Huang et al[18]	Post mortem tractography	4.7T	3D SE, 32/700 ms	7	1000 s/mm^2	0.4/0.3 mm	21 h
Kasprian et al[13]	In utero tractography	1.5T	SS-EPI, 90/1745*	32	700 s/mm^2	1.44/4.5 mm	1 min 49 s
Huang et al[19] and Song et al[21]	Postmortem tractography	4.7T†	3D SE, 66/800 ms	7	1000 s/mm^2	300–600 μm	20 h
Zanin et al[55]	In utero tractography	1.5T	SS-EPI, 105/8900 ms	12	1000 s/mm^2	2/2.2 mm	5 min 47 s
Takahashi et al[20] and Wang et al[22]	Postmortem tractography	4.7T	3D EPI, 40/1000 ms	60	8000 s/mm^2	Variable : 525/600 μm	2 h
Jakab et al[16]	In utero connectomics	1.5T	SS-EPI, 90/1745* ms	15	700 s/mm^2	1.44/4.5 mm	1 min 49 s
Marami et al[42]	In utero connectomics	3.0T	N/A/60/3–4000 ms	12	500 s/mm^2	2/2–4 mm	50–90 s
Song et al[91]	In utero + postnatal tractography (in utero is detailed here)	1.5T	SS-EPI, 90/"shortest''	16	700 s/mm^2	Variable, 2/4 mm	1 min 16 s
Lockwood Estrin et al[92]	In utero tractography	1.5T	SS-EPI, 121/8500	15	500 s/mm^2	2.3/3.5 mm	12 min

*Variable TR.
†Protocol used for fetal brains over 17 weeks of gestation.
3D SE indicates 3D spin echo sequence; SS-EPI, single-shot spin echoplanar imaging sequence.

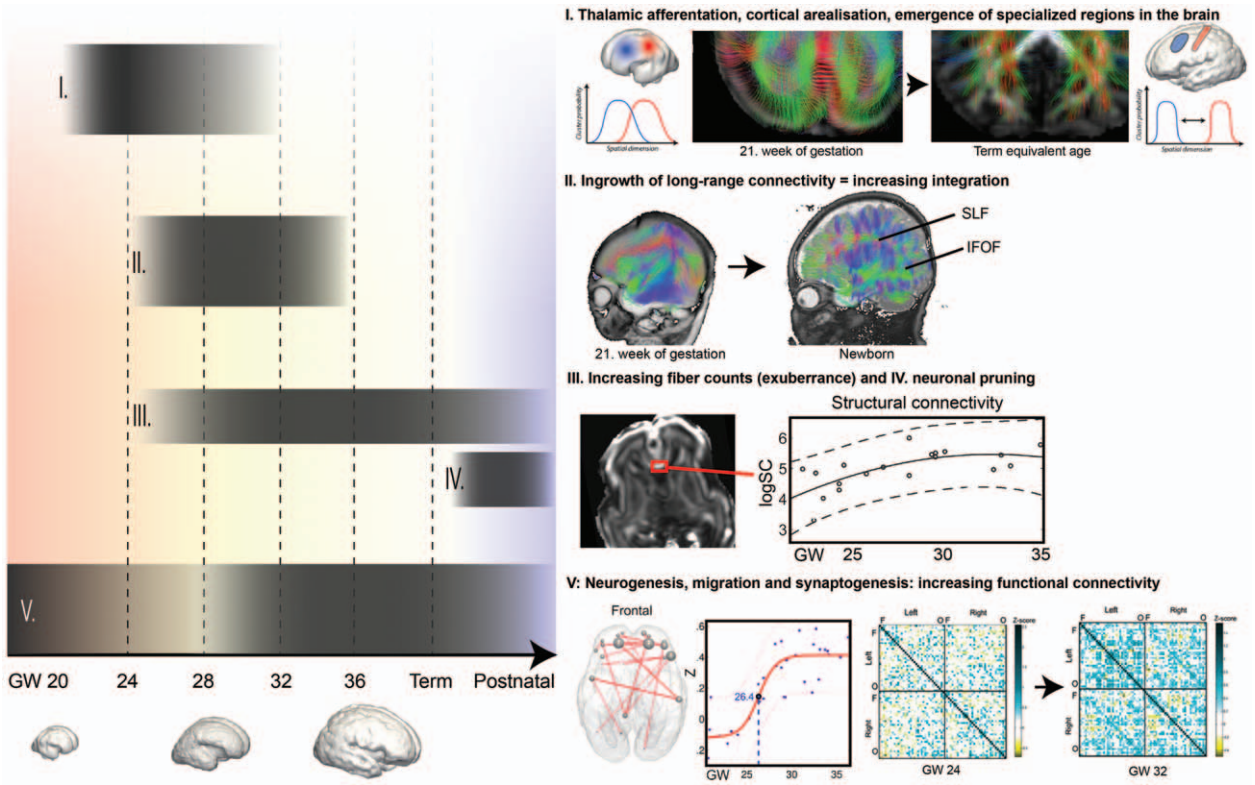

FIGURE 1. Critical steps of brain connectivity development and possible links to MRI detectable phenomena. (I) Ingrowth of thalamic afferentation and cortico-cortical connections establish circumscribed cortical areas. Axial plane images show the in utero diffusion tensor tractography of a fetus at the 21st week of gestation and the same region in a newborn. Cortical connectivity profiles become more circumscribed during the second half of gestation (left and right image panels); (II) The emergence of long-range connectivity (SLF: superior longitudinal fasciculus, IFOF: inferior fronto-occipital fasciculus) results in increasing integration. Long-rage pathways are demonstrated using whole-brain diffusion tractography in a 21-week-old fetus and a newborn; (III) Brain volume, distances and fiber length increase in parallel to axonal outgrowth and synaptogenesis, which is gradually becoming more affected by neuronal pruning (IV). Left image: axial diffusion anisotropy image of a normally developing fetus at the 21st week of gestation, right image: gestational age-related change of the structural connectivity strength of callosal connections; (V) The development of synapses in the cortical plate and late-gestational increase of synaptogenesis may trigger increasing functional connectivity. Whole-brain functional connectivity is displayed as connectivity matrices (*Z*-score) at the 24th and 32nd week of gestation.

et al[37]), in the field of in vivo neuroimaging, it is almost exclusively used to refer to the macroscopic spatial scale, meaning that the smallest possible imaging unit typically has a volume of a few cubic millimeters. High-field postmortem (ex vivo) MRI may improve spatial resolution by an order of magnitude,[38] and postmortem diffusion imaging benefits from the lack of movement and increasing signal-to-noise ratio (eg, postmortem fetal DTI data set of the Allen Brain atlas with 200 μm voxel resolution,[39] for a review on ex vivo fetal brain MRI, see Vasung et al [40]). In the time dimension, MRI most reliably characterizes the temporal dynamics of the functional connectome over the scale of minutes (dynamic functional connectivity analysis).

Fetal MRI no longer requires the sedation of the mother or the fetus, which makes retrospective correction of fetal or maternal movements challenging: both in utero dMRI and fMRI suffer from multiple sources of motion. In addition to fetal motion, maternal breathing contributes to the variability in fetal MR data. To improve the data usability, various post processing techniques have been recently proposed to correct for motion, and to reconstruct fetal MRI and dMRI data in higher resolution despite subject motion.[41–43]

DEVELOPMENTAL PATHOCONNECTOMICS: BIOLOGICAL BACKGROUND

Developmental pathoconnectomics aims at understanding how cerebral connections and the emergent properties of the entirety of cerebral connections are affected by diseases that have putative neurodevelopmental origins. To apply this concept in practice, it is important to understand how pathological processes may interfere with the early development of the brain connectome. Five important neurodevelopmental processes are highlighted (illustrated in Table 2), with which pathologies may interfere: (I) ingrowth of thalamocortical connectivity and establishment of a *blueprint* for the connectional topography of the cortex, (II) development of long-range cortico-cortical connectivity, (III) the ''mechanistic'' growth of white matter bundles as a result of increasing axon numbers, which may indirectly impact a wider range of connectivity measures, (IV) neuronal pruning to remove excessive connectivity, and (V) neuronal migration and synaptogenesis, which may lead to the establishment of the first ''activations'' and functional connectivity detected in in utero resting-state MRI experiments.

TABLE 2. Summary of the Commonly Used In Utero fMRI Acquisition Protocols for Functional Connectivity Analysis

Study	Aim/Technique	Field Strength	Sequence, TE/TR	Number of Image Frames	Voxel Dimension and Slice Thickness	Acquisition Time
Schöpf et al[31,32]	Single subject ICA	1.5T	SS-EPI, 100–140/1000 ms	50–300	1.8/5 mm	1–3 min
Thomason et al[33]	Group ICA	1.5T	SS-EPI, 30/2000 ms	180	3.4/4 mm	6 min
(same as above)	(same as above)	3.0T	SS-EPI, 50/2000 ms	220	3.4/4 mm	7 min 20 s
Ferrazzi et al[34]	Group ICA	1.5T	SS-EPI, 50/4000 ms	100	2.5/5 mm	6 min 40 s
Jakab et al[16]	Functional connectomics	1.5T	SS-EPI, 50/1000 ms	50	1.8/5 mm	1 min
Thomason et al[80]	Functional connectomics	3.0T	SS-EPI, 30/2000 ms	343 (average)	3.4/4 mm	11 min (average)
Thomason et al,[78] Thomason et al,[88] Thomason et al,[82] Thomason et al[84]	Functional connectomics	3.0T	SS-EPI, 30/2000 ms	180–349 (average)	3.4/4 mm	11 min (average)
Seshamani et al[35]	Group ICA	1.5T	EPI, dual echo: 15.6–45/3000 ms	92	N/A/3 mm	5 min
van den Heuvel et al[81]	Functional connectomics	3.0T	SS-EPI, 30/2000 ms	360	3.4/4 mm	12–24 min

Acquisition times were either calculated as TR∗time-frames or explicitly defined in the manuscript.

3D SE indicates 3D spin echo sequence; ICA, independent component analysis; N/A, not available or not detailed in the manuscript; SS-EPI, single-shot spin echo echo planar imaging sequence.

Cortical specialization arises as a result of processes (I) and (II) and it is safe to assume that pathological processes interfering with them would lead to connectomes that are characterized by impaired community structure. Connectomic analysis often relies on predefined atlases or subdivisions (parcellations) of the brain,[8] and this parcellation likely emerges and undergoes rapid and dramatic topological changes from the 20th week of gestation until birth. During mid-gestational development in humans, the early appearing thalamic afferentation determines important aspects of the functional organization of the cortex and allows for the establishment of a connectivity *blueprint*.[44,45] The ingrowth of thalamic afferents at the beginning of the second trimester of gestation is therefore central in determining cortical and subcortical topography, which may in turn affect connectivity metrics that reflect functional segregation in the brain.[3] One way to portray this change would be to carry out data-driven (eg, connectivity-based[1]) parcellation of the developing fetal cortex based on DTI or fMRI data. To the best of our knowledge, no studies to date have reported longitudinal changes in the connectional topography employing this technique. The appearance of circumscribed cortical areas might be paralleled by the convergence of thalamocortical and projection fibers, which has been proved by postmortem diffusion tractography.[22] While its prenatal origins are not proven, autism spectrum disorders appear to be associated with abnormal, or more variable connectivity topography.[46–48] Association pathways between cerebral lobes are already present prenatally and right after birth (Fig. 1/II),[49] as is the efficient integration across spatially segregated, hierarchically organized networks by long-range connectivity (''small-world'' architecture).[3,50–53]

Pathological processes interfering with the formation of long-range, association connections would presumably lead to diminished integration and to the loss of the characteristic community structure seen in typically developing brains. Synapses are established in excess and their elimination continues after birth; axons are produced in exuberance.[54] Neuronal pruning during development contributes to the transient structure of the connectome. In the unmyelinated fetal brain, the complex gestational age dependent changes of the fractional anisotropy, parallel and perpendicular diffusivity of the white matter may in dMRI experiments reflect this process.[55] As a result of neuronal pruning, excessive connections are removed, which gives rise to nonlinear increase or increasing and later decreasing connectivity strengths in the developing connectome (Fig. 1/III). Disturbance to axonal development and synaptic elimination may therefore result in both under- or overconnectivity in the structural brain connectome and, most importantly, would also affect the intrinsic functional brain connectivity and the emerging functional brain connectome.

While postnatal—or rather adult age—advanced MRI gained popularity as a clinical tool before it was successfully used in neuroscientific endeavors, such a shift seems to be happening in the opposite direction for advanced fetal MRI, in particular dMRI and BOLD fMRI. Until now, attempts to describe the normally developing structural and functional brain connectome dominated the field, together with works that extrapolate knowledge from prematurely born infant brain development to in utero processes. This is well reflected by the following overview of studies on the normal and pathological connectome development.

EMERGENCE OF WHITE MATTER TRACTS

The first in utero and postmortem dMRI studies of the human fetus provided invaluable information about the development of white matter tracts as early as the second trimester of gestation.[13,19,49,56–58] Observing the timing of how white matter tracts emerge is crucial for the understanding of the dynamic maturation of the structural brain connectome, and to link its emergent properties to developmental processes. Before the 24 weeks of gestation, connectivity of the human brain is dominated by transient structures, and the established neural circuits mostly reach the intermediate zone and subplate.[59] The first connections to penetrate the cortical plate are afferents from the thalamus that were previously waiting in the subplate[38]; therefore, the ingrowth of thalamocortical connections marks the beginning of a series of events that lead to the emergence of the structural brain connectome.[60] Some of these developmental events are apparent on anatomical MRI, such as the process of cortical laminarization and the resolution of the subplate.[61]

White matter bundles show a heterogeneous pattern of their emergence. The first time point during development at which a certain tract becomes visible on DTI or histology has been detailed in previous works.[19,62–64] There is consensus that limbic tracts, in particular the fornix and cingulate bundle, are already present at the 19 weeks of gestational age. The corpus callosum emerges at the beginning of the second trimester, its maturation follows a central to peripheral pattern, with the splenium visible after the 19 weeks of gestation and the forceps minor at the 25 weeks. Projection tracts, particularly the anterior limb of the internal capsule encompassing

the anterior thalamic radiations, are visible from the 22 weeks. The first association bundles to appear are the external capsule, uncinated fasciculus, and the inferior fronto-occipital fasciculus, all present before the 22 weeks of gestation on DTI.[64] The major association tracts are detected during the third trimester of gestation[49]; however, the superior bundles, such as the superior longitudinal fasciculus or arcuate fasciculus, appear to be discontinuous until end of second trimester.

GESTATIONAL CHANGES OF THE STRUCTURAL BRAIN CONNECTOME

Normal Developmental Maturation of the Connectome

Only a few studies investigated so far how whole-brain and network-level properties of the structural brain connectome develop prenatally in normal and pathological conditions. One such work is by Song et al,[21] who carried out an in-depth analysis of the age-dependent changes of the human fetal connectome using post mortem DTI. Between the 20th week of gestation and term, network strength, global efficiency, and local efficiency increase dramatically and significantly, which is also in line with the observations of postnatal preterm brain development.[65,66] As hypothesized earlier in this review, the increasing efficiency and integration is potentially initiated by the ingrowth of long-range association fibers toward the end of gestation. Efficiency and strength appear to increase more rapidly between the 20 and 35th postmenstrual weeks than later and can increase the fiber count between certain paired regions. Surprisingly, the human fetal brain displays small-world characteristics as early as the 20th postmenstrual week; however, as the authors point out, at least some of the ''fibers'' detected at this developmental stage with DTI would be the result of the radial migrational coherence of neurons along the radial glia,[67] which means that local, short-range connectivity might be overestimated.

Pathological Connectome Development

Pathological white matter development and aberrant, macroscopically visible fiber pathways as a primary finding during prenatal screening is uncommon. One notable exception is corpus callosum agenesis (CCA). We demonstrated that in CCA, the absence of callosal connectivity affects the global organization of cerebral connections already prenatally, most likely as a result of maladaptive development.[16] Rerouting of callosal pathways may result into heterotopic axonal pathways or thickening of preexisting tracts or phylogenetically older commissures. The most common macroscopically observable heterotopic tracts are the bundles of Probst,[68] which recently have been depicted and confirmed by prenatal DTI.[15] Prenatal connectomic analysis implies that besides the presence of the Probst bundle, the CCA connectome is characterized by excessive short-range and intralobar connections.[16] It is possible that lateral callosal fibers can join corticospinal axons and result in stronger connectivity observed and relative increase of diffusion anisotropy.[69]

NORMAL AND PATHOLOGICAL FUNCTIONAL CONNECTOME DEVELOPMENT

A variety of physiological findings of prenatal neuronal activity establish the theoretical framework for studying the developing human fetal brain with fMRI and using functional connectivity analyses. Spontaneous electrophysiological activity is displayed by developing neurons that have reached their waiting compartment in the subplate.[70,71] The firing patterns of the maturing neurons gradually evolve into the characteristic trace-alternant activity later in gestation as neurons relocate to the cortical plate.[72] With progressing synaptogenesis, firing patterns may become increasingly complex and it can be assumed that recent in utero fMRI experiments that found low-frequency signal synchronicity (eg, <0.1 Hz) reflect this process indirectly.[31] While the electrophysiological development of the human central nervous system in the second trimester remains largely unexplored, evidence from prematurely born infants suggests that the EEG activity as well as functional connectivity emerge largely during the period of rapid brain growth in the third trimester of gestation, well before the acquisition of complex cognitive capacities.[73]

The following section describes the works performed to date, which examined this period of in utero neurodevelopment using advanced fetal fMRI. The first study employing stimulus-free acquisition and independent component analysis (ICA) found that resting-state networks are shaped and are detectable as early as the 20th week of gestation in utero.[31] Among the networks discovered, 3 network components were observed throughout the second and third trimesters of gestation: a bilateral frontal, bilateral occipital, and a unilateral temporal component. The networks were characterized by frequency spectra with a peak at 0.01 to 0.06 Hz, which is largely similar to those previously seen in adults. The spatial distribution of these networks and their similarity to those in preterms imaged at term equivalent age implies a preference for sensory systems,[73,74] for example, an early maturation of visual processing. The bilateral occipital and frontal component was also confirmed by a group ICA analysis of 16 fetuses by Ferrazzi et al.[34] Visual processing and cortical control of eye movement emerges prenatally, as shown by the presence of reproducible networks that coappear with eye movements in the fetus.[75] It is also noteworthy that a high number of unilateral networks were found, of which only the unilateral temporal component was reproducibly seen across the population.[31] Thomason et al[33] utilized a similar, ICA-based approach, but on the group level, revealing significant and reproducible cross-hemispheric networks in approximately 50% of all cortical regions investigated. The functional connectivity strength and gestational age were significantly correlated in the vast majority of bilateral networks and a medial-to-lateral developmental gradient of functional connectivity was evident, confirming previous findings in premature newborns.[76]

Network-level analysis and graph theory provide a more profound understanding of how the emergent properties of the functional brain connectome develop and parallel the anatomical changes of the developing fetal brain. Our group studied the emergence of fetal brain functions in a population that included fetuses between the 21 and 32nd week of gestation,[77] revealing increasing functional connectivity in various long- and short-range functional brain connections during mid-gestation. According to these findings, functional connectivity increases nonlinearly and the timing of ''rapid expansion'' varies between brain regions. Posterior and central brain regions undergo this rapid functional connectivity increase 2 weeks before the frontal and parietal regions, presumably following a sensory to higher level association trajectory. This observed pattern partially matches the sequence of phylogenetic development of cortical functions: the increased functional synchronicity in fronto-parietal brain areas appear to emerge later compared with the synchronicity in somatosensory and visual regions. With increasing gestational age, the functional connectome is characterized by significantly more long-range connections, and the key contributors to the default mode network, such as caudal posterior cingulate cortex and medial prefrontal cortex, display increased connectivity in older fetuses.[78] These characteristic changes and developmental gradients affect the regional and global properties of the functional brain connectome.[79] With increasing gestational age, intermodule connection strength appears to increase, modularity decreases, modules begin to overlap with known functional systems, and the

posterior cingulate cortex in the default mode network shows increased negative functional connectivity with other brain areas.[80] The fetal connectome is also characterized by an increasing proportion of brain regions attaining a central role and forming hubs: putative hubs were identified in visual, motor, and association areas in a study by van den Heuvel et al.[81]

These results provide further support for the hypothesis that the human brain already prepares for higher order cognitive processing prenatally, and neural substrates serving these functions are in place at the time of birth. From the developmental pathoconnectomics perspective, this has important implications. Cerebral injury during the last trimester of gestation may profoundly disturb the development of the structural and functional brain connectome, disrupting the establishment of the densely connected hubs serving higher order cognition. It is therefore an important endeavor to relate prenatal findings to postnatal outcome to test this hypothesis and uncover the clinical and neurological effects associated with disturbed development of the fetal connectome. An initial exploration of this question was reported by Thomason et al,[82] who found that variation in the human fetal functional connectome correlates with infant motor ability. The sex of the fetus appears to interact with the functional connectivity—motor outcome correlation, and mild sex differences in the development of the functional connectome development were also revealed by the same research group.[83] Other factors, such as prenatal exposure to lead, may also result in brain connectivity impairments.[84]

There is overwhelming evidence that cerebral white matter development after premature birth is impaired, subsequently leading to pervasive changes in the brain connectome,[65] and connectivity has been suggested as a viable marker of mental development.[85,86] The term ''encephalopathy of prematurity'' comprises a complex amalgam of cerebral injury (primary destructive disease) and secondary maturational and trophic disturbances.[87] While the causes of preterm labor and premature birth are multifactorial and depend highly on the (mainly maternal) risk factors and gestational age, limited evidence exists from connectomic research for a possible common pathway of impaired neurodevelopment preceding premature birth. An analysis of in utero intrinsic functional brain connectivity of fetuses born prematurely revealed that the strength of functional connectivity in the left language-related regions was related to gestational age at delivery.[88] This finding may support emerging evidence that fetal-maternal stress leads to pervasive changes in the cerebral connectome,[89] which could be depicted prenatally using advanced fetal MRI.

PRENATAL CONNECTOME IMAGING: CHALLENGES

The major challenges in fetal dMRI, fMRI, and the corresponding analyses of structural and functional connectomes include technical difficulties relating to data acquisition, fiber tractography as well as the interpretation of findings in light of the differences between fetal and adult anatomy and tissue microstructural properties. This inherently leads to less precise estimates of whole-brain connectomic measures. Moreover, increased variability is introduced by the rapid temporal dynamics of connectome development during development, necessitating additional attention and care with statistical inferences on such data.

The clinically applicable MRI hardware designs limit the spatial precision and thus image resolution during dMRI and fMRI. The models that establish a relationship between the MRI signal and the underlying fiber population (eg, diffusion tensor) remain rather simplistic estimates of the vastly complex axonal anatomy within an imaging unit, and they do not reveal the polarity of connections (afferent vs efferent) or synaptic relationships. A further limiting factor restricting the faithful portrayal of neurodevelopment is the difficulty in locating cortical layers, which is even more problematic in the fetal brain due to its smaller size.

It is important to call attention to the fact that dMRI-based depictions of the macroscale connectome are likely dominated by false-positive connections,[25] and network properties, such as the hub or rich-core structure, show low correspondence to ground truth anatomical tracer results in animal studies.[90] According to reproducibility studies,[58,91] the ''detectability'' of fibers using in utero MRI is moderate to high; however, the low reproducibility of brainstem and association fibers call for careful interpretation of such results. On the basis of FA maps, Lockwood Estrin et al[92] reported a tract visibility of 81% to 92%, which is lower than the same number in preterm infants of the same age, emphasizing the fact that the in utero setting yields suboptimal results to ex utero MRI. This poses a general challenge to the interpretability of connectomic results. Reproducibility studies using clinical in utero DTI sequences revealed a ''moderate'' detectability and within-subject variability of dMRI-based metrics 2 to 4 times worse than the corresponding metrics acquired postnatally. Song et al[91] performed a pre- to postnatal comparison of fiber tracts' detectability and found moderate degrees of accuracy (67% to 75%) for the 4 segments of the corpus callosum and moderate to high degrees of accuracy (75% to 92%) for the corticospinal tracts. This is in line with the findings of our research group on tract detectability for the commissural and project fibers.[58] Nevertheless, it is safe to assume that more precise diffusion models, such as higher order spherical harmonics reconstruction and tractography approaches, would increase the reliability of the approach.[93,94]

In contrast to dMRI-based axonal pathway visualization in the adult brain,[23] the exact microstructural correlates of diffusion anisotropy remain unexplored in the unmyelinated and immature fetal brain,[13] and we lack high-resolution validation data of brain connectivity. Transient cellular components, such as radial glia and the migrational coherence of neuronal populations, may contribute to in vivo fetal DTI findings.[67,95] In the developing fetal brain, all major fiber bundles have to find their way through periventricular structures, which are transient crossroads facilitating radial and tangential migration.[96] To depict fiber bundles, such as association fibers or commissural fibers, higher angular resolution dMRI is needed with more diffusion-weighting directions or b-values, which allows for the modeling of crossing fiber populations within each voxel,[97] but would inherently result in longer imaging times and increase the likelihood of fetal motion. Possible ways to overcome the limitations of dMRI and tractography for more reliable connectome mapping would include (1) informing the tractography algorithms with microstructural properties learned from ground truth microscopic or postmortem data[98,99] and (2) cross-validating the resulting fiber pathways using neuronal tracers, which has only been done for adult human or primate brains,[100–104] or in mice.[105] The intrauterine position of the fetus is also relevant for the quality of dMRI source data,[13,106] and the heterogeneous chemical composition of the pelvis of the mother induces considerably larger artefacts during echo-planar imaging in utero than ex utero.

As with all BOLD fMRI studies, the interpretability is somewhat limited by the assumption that neuronal activity and the activity-induced hyperemia are tightly coupled, and that the characteristics of the hemodynamic response function (HRF) are true under the given experimental conditions or in a given pathology. This is a profound challenge for the fetal brain, as our current knowledge for the development of the HRF mostly stems from preterm studies,[107] which reflect a nonphysiological developmental trajectory. In most postnatal studies, the ''resting'' condition is well controlled during fMRI measurements; however, it is not directly possible to control this in fetal imaging experiments, and it is likely that the

changing brain states and the presence of sensory inputs, such as sounds and maternal movements, influence the functional connectivity results. Fetal behavioral states are known to show 2 characteristic patterns: periods of ''quiet sleep'' or ''quiet awake,'' in which fetal eye movements are different.[108] Besides controlling for fetal eye movements, a possible hypothetical solution would be dynamic functional connectivity analysis and the categorization of brain states.[109] An increasingly complex challenge is caused by the fact that fetal head motion may induce spurious errors in the functional connectome, and head motion patterns are to a certain degree gestational age-dependent, confounding the interpretation of longitudinal analyses and posing a general statistical challenge during group comparisons.

A crucial part of connectomic analysis is the spatial standardization of DTI or fMRI data, that is, to achieve anatomical correspondence between subjects, which ensures reliable and meaningful results from group-level statistical analysis. This is typically done by coregistering data with imaging templates that are specific for a developmental stage. Second, spatiotemporal atlases may serve as references of normal neurodevelopment. Currently, a group-wise DTI atlas has been published that would be an invaluable resource for future structural connectomic studies.[63] Although there is a growing body of knowledge describing the organization of the adult brain into functional zones or zones based on cytoarchitecture, we currently lack similar atlases of the developing human fetal brain, which inherently limits the crucial parcellation (or ROI) definition step in connectomic analysis. Data-driven methods or anatomically unconstrained approaches for parcellating the brain would overcome this limitation in the fetal brain.

CONCLUSION

In the critically sensitive mid-gestational phase of development, structural and functional assessment of the fetal brain would open a novel window into prenatal diagnostics and prognostics, and elucidate the diseases that may interfere with synapse formation, white matter maturation, neuronal migration, and cortical lamination.[110–112]

The recent in vivo, in utero studies presented in this review deliver novel support for the exciting hypothesis that the late gestational period might be a potential window of vulnerability. Impairments during this time may interfere with the development of neural substrates serving higher cognition, which may only manifest in later life or only result in mild cognitive deficits. This might be important, as many (or the majority of) mental illnesses can be conceptualized as neurodevelopmental disorders in which the impairment of brain connectivity development—'' miswiring''—is the single most important step during the disorder pathogenesis.[113–115] The fact that the peak prevalence of mental disorders in the first 2 decades of life overlaps with an active period of brain connectivity development may support the developmental origins of disorders, such as schizophrenia or autism.[116] As the majority of the brain's connections are established prenatally, it is likely that some mental disorders have prenatal origins.[89,117] Pre- and perinatal events leading to disrupted connectivity may therefore impact the development of cognitive capacities and behavior, which may only be manifested in later life. Proving this assumption remains an elusive effort for which it is critical to understand the various steps of brain connectivity development, and to explore the way developmental processes may give rise to phenomena, which can even be detected prenatally using the currently available neuroimaging techniques.

Advanced fetal MRI has the potential to become a tool for connectomic imaging, but for this endeavor, optimized imaging and post processing technologies are indispensable. As a first step, we need to establish the utility of these techniques as clinical tools by validating the imaging findings against the clinical-developmental outcome. Novel insights into brain connectivity development will have the potential to be utilized during prenatal counselling and for designing new, early therapeutic strategies such as sensory stimulation,[118,119] music therapy,[120] neuroprotection in preterm born infants,[121] and in understanding the possible prenatal origins of connectivity impairments in congenital disorders,[122] in prematurity,[85,123–125] autism spectrum,[126] or schizophrenia.[127]

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